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Cardiac Electrophysiology I

1735-Pos

Action Potential Duration Adaptation and Reverse-Rate Dependency in Human Ventricular Myocytes: Insights from a Computer Model

Eleonora Grandi, Donald M. Bers.
University of California Davis, Davis, CA, USA.

We recently developed and validated a novel mathematical model for Ca handling and ionic currents in human ventricular myocytes that is more robust than previous models in recapitulating relative contributions of various repolarizing K currents, and in describing Ca cycling processes (Biophys J. 2009;96(3) S1:664a-665a). Here, we present intriguing results on some emergent properties of this model. With increasing pacing frequency, AP shortening and parallel increases in intracellular [Ca] and [Na] are predicted. Remarkably, accumulation of $[Na]_i$ at fast rates predominates in the AP shortening (e.g. vs. K current changes), due to outward shifts in Na-pump (I_{NaK}) and Na-Ca exchange (I_{NaCa}) currents. Indeed, when clamping $[Na]_i$ to prevent Na accumulation, APD does not change with heart rate. No APD adaptation occurs when we prevent only I_{NaK} and I_{NaCa} from sensing the $[Na]_i$ rise (i.e. neither fast nor background Na currents contribute), and simulations indicate that I_{NaK} is dominant over I_{NaCa} in this effect. Moreover, acute Na-pump blockade is expected to causes gradual AP shortening as seen experimentally, that is secondary to gradual Na accumulation (after instantaneous APD prolongation due to block of outward I_{NaK}). We speculate that the increased $[Na]_i$ seen in heart failure may limit the AP prolongation that is caused by reduced K currents and increased late Na current. Our model (uniquely among human AP models) recapitulates reverse-rate dependence of APD upon I_{Kr} block, e.g. drug-induced AP prolongation is larger at slow stimulation rates. Simulation indicates that this is not due to frequency dependent properties of repolarizing currents (e.g. I_{Ks}), but is ''intrinsic'' to the system. That is, when AP repolarization is slower (at lower frequency with smaller net repolarizing current) any given current change (I_{Kr} block) causes a larger APD change.

1736-Pos

Effects of Stochastic Channel Gating and Stochastic Channel Distribution on the Cardiac Action Potential

Jan P. Kucera, Enno de Lange.
University of Bern, Bern, Switzerland.

Cardiac ion channels exhibit stochastic conformational changes that determine their open-close gating behavior, leading ultimately to the action potential (AP). However, in computational models of conduction, ion currents are usually represented deterministically. Moreover, the natural intercellular variability of the number of membrane and gap junctional channels is never considered. Our aim was to quantify the effects of stochastic current fluctuations and channel distributions on AP duration (APD) and intercellular conduction delays (ICDs) using a ventricular cell model (Rudy et al.) with Markovian formulations of the principal ion currents (I_{Na}, $I_{Ca,L}$, I_{Kr}, I_{Ks} and I_{K1}). Stochastic channel transitions were simulated explicitly and channel counts were drawn randomly from Poisson distributions.

In single cells paced at 1 Hz, stochastic channel gating generated APD variability (APD = 143 ± 1.8 ms) with a coefficient of variation (CVar) of 1.3%. APD variability decreased at higher pacing frequencies. I_{Ks} fluctuations contributed most (85%) to APD variance, followed by those of $I_{Ca,L}$ and I_{Kr} (12% and 2%, respectively). Poissonian channel distribution induced APD variability with a CVar of 0.65%. In cell strands, the CVar of APD was strongly decreased by intercellular coupling. During conduction, stochastic channel gating generated ICD variability with a CVar of 0.25%. Reduction of I_{Na} or gap junctional coupling slowed conduction, but did not increase the CVar of ICDs above 1%. Poisson distribution of membrane channels exerted a similar small effect. However, during strong gap junctional uncoupling (60-200 channels/junction, conduction velocity <1 cm/s), Poisson distribution of gap junction channels resulted in a large ICD variability (>20%), highly heterogeneous conduction patterns and conduction blocks.

Therefore, the variability of the number of channels in different gap junctions contributes to the heterogeneity of conduction patterns observed previously in experiments in cardiac tissue with altered intercellular coupling.

1737-Pos

Comparison of the Effects of the Transient Outward Potassium Channel Activator NS5806 on Canine Atrial and Ventricular Cardiomyocytes

Kirstine Calloe[1], Natalie Chlus[2], Eyal Nof[3], Thomas Jespersen[1], Søren-Peter Olesen[1], Charles Antzelevitch[2], Jonathan Cordeiro[2].
[1]University of Copenhagen, Copenhagen, Denmark, [2]Masonic Medical Research Laboratory, Utica, NY, USA, [3]Sheba Medical Center Tel Hashomer, Ramat Gan, Israel.

Objective: NS5806 activates the transient outward potassium current (Ito) in canine ventricular cells. We compared the effects of NS5806 on canine ventricular versus atrial tissues and myocytes. Methods: NS5806 (10 μM) was evaluated in arterially-perfused canine right atrial and left ventricular wedges. Atrial and ventricular epi- and endocardial cells were isolated by enzymatic dissociation. Current and voltage-clamp recordings were made in the absence and presence of NS5806. Results: In ventricular wedges NS5806 increased phase 1 repolarization in epi- and midmyocardial cells. A minor effect on conduction and upstroke velocity also was observed. In contrast, application of NS5806 to atrial preparations slowed upstroke velocity and reduced excitability, consistent with sodium channel block. In ventricular myocytes, NS5806 increased the magnitude of Ito by 80% and 16% in epi and endo, respectively (at +40 mV). In atrial myocytes, NS5806 increased peak Ito by 25% and had no effect on the sustained pedestal current, IKur. INa density in atrial myocytes was nearly 100% greater than in endocardial myocytes. NS5806 caused a negative shift in steady-state mid-inactivation (V1/2) for both cell types (73.9 ± 0.27 to −77.3 ± 0.21mV for endocardial and −82.6 ± 0.12 to −85.1 ± 0.11mV for atrial cells). The shift in V1/2 resulted in a reduction of INa in both cell types. However, the more negative V1/2 in atrial cells suggests that atrial cells lose excitability at more depolarized voltages than endocardial cells which may explain the greater reduction of excitability in atrial vs ventricular wedges by NS5806. Conclusion: NS5806 produces a prominent augmentation of Ito with little effect on INa in the ventricles, but a potent inhibition of INa with little augmentation of Ito in atria.

1738-Pos

Intracellular Zn^{2+} Release Modulates Cardiac Ryanodine Receptor Function and Cellular Activity

Esma N. Zeydanli[1], Erkan Tuncay[1], Aytac A. Seymen[1], Ayca Bilginoglu[1], Nazli Sozen[1], Mehmet ugur[1], Guy Vassort[2], **Belma Turan**[1].
[1]Ankara University School of Medicine, Ankara, Turkey, [2]INSERM U-637 Physiopathologie Cardiovasculaire, Montpellier, France.

Several Ca^{2+}-binding proteins bind also Zn^{2+}, suggesting that Zn^{2+} can modulate the structure and function of many proteins involved in heart function. We first investigated intracellular Zn^{2+} homeostasis and its possible role in cardiac excitation-contraction (EC)-coupling by using confocal microscopy in adult rat cardiomyocytes loaded with either Zn^{2+}- or Ca^{2+}-specific dye, FluoZin-3 or Fluo-3, respectively. The local ionic releases (sparks) recorded in FluoZin-3 loaded cells were significantly smaller, shorter and less frequent than those of the Fluo-3 loaded cells under control resting conditions. Following 1-μM zinc-pyrithione exposure, the amplitude of the FluoZin-3 sparks increased by 35% leaving Ca^{2+}-sparks unaffected, and a 10-mV leftward shift was observed in the L-type Ca^{2+}-current (I_{Ca})-voltage relation without significant effect on maximal I_{Ca} density. Applications of either caffeine or ryanodine, and either a mitochondrial (MT) protonophore or a MT complex I inhibitor suggested that both sarcoplasmic reticulum and mitochondria are intracellular Zn^{2+} pools. Our western-blot data further showed that there are correlations between the intracellular Zn^{2+} level and the hyperphosphorylation levels of RyR2 and CAMKII as well as with total PKC activity. Additionally, hyperphosphorylation levels of both ERK-1 and NF-κB also showed a strong dependency on internal Zn^{2+}-level. In conclusion, intracellular Zn^{2+} might have an important role in the regulation of heart function including transcription and gene expression, implying that intracellular Zn^{2+} not only has a role in EC-coupling but it is also a major intracellular second messenger in cardiomyocytes.

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Uneven Expression Patterns of KCNQ1 and KCNE Subunits in the Heart Impact on the Function of Slow Delayed Rectifier (I_{Ks}) Channels

Dimitar P. Zankov[1], Min Jiang[1], Mei Zhang[1], Yu-Hong Wang[1], M. Horie[2], F. Toyoda[2], H. Matsuura[2], Gea-Ny Tseng[1].
[1]Virginia Commonwealth University, Richmond, VA, USA, [2]Shiga University of Medical Science, Otsu, Japan.

Background: I_{Ks} has 2 major components: pore-forming (KCN)Q1 channel & regulatory (KCN)E1 subunits. Human heart expresses other members of the KCNE family (E2 - E5) that can all associate with Q1 & confer distinctly different channel phenotypes. The expression patterns of Q1 & different KCNE subunits in the heart, and their relation to the I_{Ks} channel function, is not clear. **Methods:** We use immunoblotting to quantify the protein levels of Q1 & E1 - E3 in different regions of the heart, and use patch clamp to quantify I_{Ks} current density and gating kinetics in left atrial (LA) and ventricular (LV) myocytes. We use the guinea pig model because of the robust cardiac I_{Ks}. **Results**: Immunoblot data: (1) Q1 protein is more abundant in atria (A) than in ventricles (V) (immunoblot densitometry ratio of A:V ~ 1:0.4), (2) E1 & E2 proteins are more

abundant in V than in A (E1 ratio ~ 1:2 - 3; E2 ratio ~ 1:2), (3) the E3 Ab recognizes a specific 20 kDa band in A but not in V. Patch clamp data: (1) I_{Ks} current density is much higher in LA than in LV myocytes, (2) I_{Ks} half-maximal activation voltage is more negative in LA than in LV myocytes, (3) I_{Ks} activates faster in LA than in LV myocytes. **Conclusion**: Q1 and E3 are more abundant in A than in V, while E1 & E2 have the opposite expression pattern. The uneven protein expression patterns can enhance I_{Ks} contribution to atrial action potential repolarization by generating a higher I_{Ks} density, that can reach a higher degree of activation in the action potential plateau range, than its counterpart in the ventricles.

1740-Pos

CaMKII Regulation of the Dynamic L-Type Ca^{2+} Current and Na^{+}/Ca^{2+} Exchange Current During Action Potential in Cardiac Myocytes

Tamas Banyasz[1,2], Leighton T. Izu[1], **Ye Chen-Izu[1]**.
[1]UC Davis, Davis, CA, USA, [2]Univ. of Debrecen, Debrecen, Hungary.

The L-type Ca^{2+} current ($I_{Ca,L}$) and the Na^{+}/Ca^{2+} exchange current (I_{NCX}) are the main inward currents that contribute to the depolarization during cardiac action potential (AP) plateau and later phases. Pathological changes of $I_{Ca,L}$ or I_{NCX} can cause early or delayed afterdepolarization (EAD, DAD). The steady-state kinetics of $I_{Ca,L}$ and I_{NCX} have been characterized in previous studies. However, the non steady-state dynamics of $I_{Ca,L}$ and I_{NCX} during the AP cycle still remain unclear. Here we report the new data on the dynamic $I_{Ca,L}$ and I_{NCX} during the cell's AP recorded using the *self AP-clamp* method. **Results:** (***1***) The I_{NCX} was isolated using its specific inhibitor SEA0400 at 3 μM. The data show that I_{NCX} is an inward current during most of the AP cycle. Importantly, I_{NCX} is the dominant contributor to a pronounced inward *foot current* at AP phases-3&4. This foot current is important because it depolarizes the cell at the late phases of AP and directly links to EAD or DAD. (***2***) Furthermore, the foot current is abolished by Ca^{2+}-calmodulin dependent kinase II (CaMKII) inhibition. (***3***) The $I_{Ca,L}$ was isolated using 10 μM nifedipine. The dynamic $I_{Ca,L}$ takes the form of a spike at AP phase-1 and a dome at phase-2. (***4***) Both $I_{Ca,L}$ and I_{NCX} during the AP are affected by using EGTA to buffer the SR Ca^{2+} release and prevent the CaMKII activation. **Conclusion**: Here we show for the first time the dynamic $I_{Ca,L}$ and I_{NCX} currents during the cell's AP in physiological milieu. CaMKII modulation of the foot current might explain, in part, the effect of elevated CaMKII activity on promoting arrhythmias in the hypertrophied and failing hearts.

1741-Pos

KCNQ1/KCNE1 K+ Channels Associated with Long QT Syndrome are Expressed in Early Stage Human Embryonic Stem Cell-Derived Cardiomyocytes

Cecile Terrenoire[1], George K. Wang[1], Eric Adler[2], Aaron D. Kaplan[3], Kevin J. Sampson[1], Jonathan Lu[1], Robert S. Kass[1].
[1]Columbia University, New York, NY, USA, [2]OHSU, Portland, OR, USA, [3]Mount Sinai School of Medicine, New York, NY, USA.

Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) are not only a potential source of functional cardiac tissue that can be utilized as a drug screening platform or agents for cell-based therapy but also offer great potential in studies of heritable cardiac arrhythmias known as channelopathies. Many major cardiac ion channels have been reported to be expressed in hESC-CMs. However, the presence of KCNQ1/KCNE1 (I_{Ks}) K^{+} channels critical to cardiac repolarization particularly during sympathetic nerve stimulation and associated with the most common variant of congenital Long QT syndrome (LQT1), to date has not been reported. Here we report investigation of the cellular electrophysiological properties of hESC-CMs during the first 34 days of cytokine directed differentiation with a focus on I_{Ks} channels. All beating hESC-CMs studied had action potentials with cardiac phenotypes and expressed L-type calcium channels (n=26) and pacemaker channels (n=27) while 68% of cells (n=11 out of 16) expressed I_{Kr}, the potassium current associated with LQT2, defined as E4031-sensitive outward current measured during prolonged depolarization. I_{Ks}, the potassium current associated with LQT1, was identified by its biophysical and pharmacological properties: recorded in 29% of cells (n=5 out of 17), I_{Ks} was defined as an outward current slowly activating during prolonged depolarization, insensitive to E4031 (5 μM) and blocked by Chromanol 293B (30 μM). qPCR experiments confirmed the presence of I_{Ks} channels α- (KCNQ1) and β- (KCNE1) subunits in these hESC-CMs. This is the first report of I_{Ks} channel expression in hESC-CMs providing strong evidence in support of their use in mechanistic and pharmacological investigations of LQT1 and other heritable arrhythmia syndromes linked to mutations in the genes coding for I_{Ks} channel subunits and/or accessory proteins.

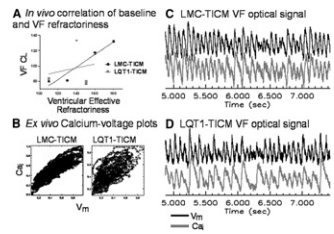
1742-Pos

LQT1 Genotype in Tachypaced Cardiomyopathy Causes Discordance of Baseline and VF Refractoriness

Ohad Ziv[1], Emily Lau[1], Lorraine Schofield[1], Lenny Chaves[1], Xuwen Peng[2], Bum-Rak Choi[1], Gideon Koren[1].
[1]Rhode Island Hospital, Providence, RI, USA, [2]Pennsylvania State University, Hershey, PA, USA.

Reduction of the slow outward rectifier (I_{ks}) and calcium disregulation accompany tachypacing induced cardiomyopathy (TICM). While TICM I_{ks} downregulation prolongs APD, its effect on refractoriness during VF is less clear. We used a transgenic rabbit model of Long QT 1 (LQT1) to investigate the effect of loss of I_{ks} on VF refractoriness in TICM.

Five LQT1 and littermate control rabbits underwent rapid RV pacing followed by *in vivo* electrophysiological studies and VF inductions. Dual voltage-calcium epicardial optical mapping was performed on whole hearts at baseline and in VF. *In vivo,* a strong correlation for ventricular effective refractoriness and VF interval was seen in LMC-TICM, but not in LQT1-TICM ($r = 0.83$ vs $r = 0.36$; $p<0.05$). Optical mapping demonstrated APD prolongation in LQT1-TICM compared to LMC-TICM (224 ± 18ms vs. 191 ± 15ms), but surprisingly higher VF frequencies in LQT1-TICM (15.7 ± 0.8 vs 12.6 ± 0.7Hz; $p<0.05$). In spatial VF frequency maps, LMC-TICM showed a negative VF frequency-APD map correlation (-0.43 ± 0.24), while LQT1-TICM demonstrated a paradoxical positive correlation (0.22 ± 0.14; $p<0.05$). Calcium-voltage discordance was increased in LQT1-TICM compared to controls (see fig). LQT1-TICM leads to dissociation between baseline and VF refractoriness demonstrating high frequency VF associated with calcium-voltage discordance.



1743-Pos

Unique Molecular Profile of Transient Outward Potassium Current (I_{to}) Subunits in Cardiac Purkinje Fibers

Ling Xiao, Balazs Ordog, Maya Marmabachi, Ange Maguy, Stanley Nattel.
Montreal Heart Institute, Montreal, QC, Canada.

Background and objective: Cardiac Purkinje-fiber (PF) tissue plays a key role in conduction and arrhythmogenesis. The transient outward K^{+}-current (I_{to}), an important cardiac repolarizing conductance, has unusual kinetic and pharmacological properties in PF cells (PCs), suggesting a distinct and presently-unknown molecular basis. The present study addressed the differential expression of putative I_{to}-subunits in PF versus left-ventricular (LV) myocardium.

Methods: I_{to} was recorded with whole-cell voltage-clamp at 36°C from isolated PCs or LV cardiomyocytes before and after the K^{+}-channel blocker TEA. The regional mRNA expression-levels of I_{to} α-subunit (Kv4.3, Kv3.4) and β-subunit (KChIP2, NCS-1, Kvβ1, KChAP, KCNE1-5, and DPPX_S/_L) candidates were determined by real-time PCR.

Results: I_{to} from PCs was more sensitive to TEA than LV: 10 mM TEA reduced I_{to} by $53 \pm 10\%$ ($N = 5$) in PCs versus $-4 \pm 8\%$ ($N = 5$) in LV cells, $P<0.01$. The mRNA levels of I_{to} α-subunits Kv4.3 and Kv3.4 were significantly higher (by about 2.7 and 159-fold respectively: e.g., epicardium versus PF 1.67 ± 0.38 vs 3.58 ± 1.14 ΔΔ-Ct units, $P<0.05$ for Kv4.3, 0.0001 ± 0.0001 vs 0.044 ± 0.020, $P<0.05$ for Kv3.4; $N = 9$/group) in PF-tissue than in LV. KChIP2 was much richer in LV epicardium (2.91 ± 0.73) and midmyocardium (0.92 ± 0.26) than in LV endocardium (0.09 ± 0.02, $P<0.01$) and PF (0.07 ± 0.03, $P<0.01$). NCS-1 was abundantly expressed in PF-tissue (1.95 ± 0.68), at about 400×LV values (epicardium 0.10 ± 0.03, midmyocardium 0.16 ± 0.07, endocardium 0.15 ± 0.04). KCNE1 and KCNE3-5 mRNA levels were significantly higher (eg, by 2.8, 2.9, 3.4and 6.0-fold vs epicardium) in PF than in all LV zones, whereas Kvβ1, KCNE2, KChAP and DPPX_S/_L subunits were similarly expressed among these four regions.

Conclusion: Cardiac PF-tissue has a unique expression profile of I_{to}-subunits that may account for its unusual properties. Expression studies are under way to determine the precise biophysical mechanisms.

1744-Pos

Decreased Phosphorylation of the Gap Junction Protein Connexin43 and Increased Anisotropy of Conduction as a Consequence of Myofilament Ca2+ Sensitization

Raghav Venkataraman[1], Sung In Kim[1], Veniamin Y. Sidorov[1], James D. Potter[2], Bjorn C. Knollmann[1], Franz Baudenbacher[1], **Sabine Huke[1]**.
[1]Vanderbilt University, Nashville, TN, USA, [2]University of Miami, Miami, FL, USA.